

# A Fast Template Based Heuristic For Global Multiple Sequence Alignment

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## Abstract

Advances in bio-technology have made available massive amounts of functional, structural and genomic data for many biological sequences. This increased availability of heterogeneous biological data has resulted in biological applications where a multiple sequence alignment (msa) is required for aligning similar features, where a feature is described in structural, functional or evolutionary terms. In these applications, for a given set of sequences, depending on the feature of interest the optimal msa is likely to be different, and sequence similarity can only be used as a rough initial estimate on the accuracy of an msa. This has motivated the growth in template based heuristics that supplement the sequence information with evolutionary, structural and functional data and exploit feature similarity instead of sequence similarity to construct multiple sequence alignments that are biologically more accurate. However, current frameworks for designing template based heuristics do not allow the user to explicitly specify information that can help to classify features into types and associate weights signifying the relative importance of a feature with respect to other features, even though in many instances this additional information is readily available. This has resulted in the use of ad hoc measures and algorithms to define feature similarity and msa construction respectively.

In this paper, we first provide a mechanism where as a part of the template information the user can explicitly specify for each feature, its type, and weight. The type is to classify the features into different categories based on their characteristics and the weight signifies the relative importance of a feature with respect to other features in that sequence. Second, we exploit the above information to define scoring models for pair-wise sequence alignment that assume segment conservation as opposed to single character (residue) conservation. Finally, we present a fast progressive alignment based heuristic framework that helps in constructing a global msa by first constructing an msa involving only the informative segments using exact methods, and then stitch into this the alignment of non-informative segments constructed using fast approximate methods.

*Key words:* Analysis of algorithms; Bioinformatics; Computational Biology; Multiple Sequence Alignment; Template Based Heuristics

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## 1. Introduction

A global multiple sequence alignment (msa) [7, 17, 29] of a set  $\mathcal{S} = \{S_1, S_2, \dots, S_k\}$  of  $k$  related protein sequences is a way of arranging the characters in  $\mathcal{S}$  into a rectangular grid

of columns by introducing zero or more spaces into each sequence so that similar sequence features occur in the same column, where a feature can be any relevant biological information like secondary/tertiary structure, function, domain decomposition, or homology to the common ancestor. The goal in attempting to construct a global msa is either to identify conserved features that may explain their functional, structural, evolutionary or phenotypic similarity, or identify mutations that may explain functional, structural, evolutionary or phenotypic variability.

Until recently, sequence information was the only information that was easily available for many proteins. So, the measures that were used to evaluate the quality (accuracy) of a msa were mostly based on sequence similarity. The *sum of pairs score* (SP-score) and *Tree score* were two such measures that were widely used. For both these measures, the computation of an optimal msa is known to be NP-Complete [54]. So, in practice most of the focus is on designing fast approximation algorithms and heuristics. From the perspective of approximation algorithms, constant polynomial time approximation algorithms are known for the SP-score [17,55] and polynomial time approximation schemes (PTAS) [55] are known for the Tree score. However, in practice, these approximation algorithms have large run-times that makes them not very useful even for moderate sized problem instances. From the perspective of heuristics, most heuristics are based on progressive alignment [18, 20, 48, 49, 51], iterative alignment [10, 11, 12, 14, 15, 19, 24, 47], branch and bound [45], genetic algorithms [36, 37], simulated annealing [26] or on Hidden Markov Modeling (HMM) [8, 9, 21]. For an extensive review of the various heuristics for msa construction, we refer the reader to excellent survey articles of Kemena and Notredame [25], Notredame [33, 34, 35], Edger and Batzoglou [12], Gotoh [15], Wallace et al. [52], Blackshields et al. [5].

In heuristics based on progressive alignment, the msa is constructed by first computing pair-wise sequence distances using optimal pair-wise global alignment scores. Second, a clustering algorithm (UPGMA or NJ [46]) uses these pair-wise sequence distances to construct a rooted binary tree, usually referred to as guide tree. Finally, an agglomerative algorithm uses this guide tree to progressively align sequences a pair at a time to construct a msa. The pair-wise global alignments scores are usually computed using a substitution matrix and a gap penalty scheme that is based on sequence similarity. ClustalW [51] was among the first widely used progressive alignment tool on which many of the current day

progressive aligners are based. In this paper, our focus is on heuristics that are based on progressive alignment mainly because in this method the computation of pair-wise sequence distances, guide tree and the choice of agglomerative algorithm for progressively pair-wise aligning sequences can be essentially split into three independent steps. This helps to provide a flexible algorithmic framework for designing simple parameterized greedy algorithms that are computationally scalable and whose parameters can be tuned easily to improve its accuracy. In addition, the alignments obtained through this approach are usually a good starting point for other popular approaches like iterative, branch and bound, and HMM. However, the progressive aligners because of their greedy approach commit mistakes early in the alignment process that are usually very hard to correct even when using sophisticated iterative aligners. This problem can be addressed if we can incorporate into the pair-wise scoring scheme the information for every pair of sites the frequency at which the residues at these sites are involved in alignments involving other sequences in  $\mathcal{S}$ . However, incorporating this information for all pairs of sites based on all sequences in  $\mathcal{S}$  is computationally infeasible.

The consistency based heuristics [6, 10, 11, 24, 28, 38, 39, 40, 42, 43, 47] tackle this problem by incorporating a larger fraction of this information at a reasonable computational cost as follows: The score for aligning residues at a pair of sites is estimated from a collection of pair-wise residue alignments named the library. The library is constituted of pair-wise alignments whose residue alignment characteristics are implicitly assumed to be similar to an optimal msa or a reference alignment that was constructed using sequence independent methods. For a given library, any pair of residues receives an alignment score equal to the number of times these two residues have been found aligned either directly or indirectly through a third residue. The Consistency based progressive aligners generally construct msas that are more accurate than the pure progressive aligners like clustalW. However, it is not very clear how to construct a library of alignments whose residue alignment characteristics are guaranteed to be similar to an optimal msa. In addition, the increased accuracy of msa of consistency based aligners comes at a computational cost that is on an average  $k$  times more than a pure progressive aligner. T-Coffee [38], ProbCons [6], MAFFT [24], M-Coffee [53], MUMMALS [41], EXPRESSO [2], PRALINE [42], T-Lara [4] are some of the widely used consistency based aligners.

Currently, advances in bio-technology have made available massive amounts of functional,

structural and genomic data for many biological sequences. This increased availability of heterogeneous biological data has resulted in biological applications where an msa is required for aligning similar features, where a feature is described in structural, functional or evolutionary terms. In these applications, for a given set of sequences, depending on the feature of interest the optimal msa is likely to be different, and sequence similarity can only be used as a rough initial estimate on the accuracy of an msa. In addition, from evolutionary studies we know that structure and function of biological sequences are usually more conserved than the sequence itself. This has motivated the growth in template based heuristics [50] that supplement the sequence information with evolutionary, structural and functional data and exploit feature similarity instead of sequence similarity to construct multiple sequence alignments that are biologically more accurate. In these methods, each sequence is associated with a template, where a template can either be a 3-D structure, a profile or prediction of any kind. Once a template is mapped onto a sequence, its information content can be used to guide the sequence alignment in a sequence independent fashion. Depending on the nature of the template one refers to its usage as structural extension or homology extension. Structural extension takes advantage of the increasing number of sequences with an experimentally characterized homolog in the PDB database, whereas homology extension uses profiles. 3-D Coffee [3], EXPRESSO [3], PROMALS [42, 44] and PRALINE [47] are some popular aligners that are widely used tools that employ template based methods. For more details about template based methods we refer the reader to Kemena and Notredame [25] and Notredame [34].

In template based methods, we can view each template once mapped to a sequence as essentially partitioning the sequence into segments, where each segment corresponds to a feature described by the template. Then, we construct a msa by essentially aligning segments that share similar features. The current frameworks for describing templates do not allow the user to explicitly specify information that can help (i) classify features into types and (ii) associate a weight signifying the relative importance of a feature with respect to other features, even though in many instances this additional information is readily available. This has resulted in the use of ad hoc measures and algorithms to define feature similarity and msa construction respectively.

In this paper, we

- provide a mechanism where as a part of the template information the user can explicitly

specify for each feature, its type, and weight. The type is to classify the features into different categories based on their characteristics and the weight signifies the relative importance of a feature with respect to other features in that sequence.

- define scoring models for pair-wise sequence alignment that assume segment conservation as opposed to single character (residue) conservation. Our scoring schemes for aligning pairs of segments are based on segment type, segment weight, information content of an optimal local alignment involving that segment pair, and its supporting context. This is an attempt to define scoring schemes that evaluate a pair-wise global alignment through information content of a global segment alignment, where segments correspond to features within sequences. For example, in a structurally correct alignment the focus is on aligning residues that play a similar role in the 3D structure of the sequences, whereas a correct alignment from an evolutionary viewpoint focuses on aligning two residues that share a similar relation to their closest common ancestor, and in a functionally correct alignment the focus is on aligning residues that are known to be responsible for the same function. The supporting context consists of set of sequences that are known to belong to the same family (i.e. share similar structure, function or homology to a common ancestor) as the given sequence pair and can help determine to what extent the alignment of the features in that pair-wise alignment is consistent with the alignment of these features with other sequences in the family.
- present a fast progressive alignment based heuristic that essentially constructs global msa by first classifying segments into informative or non-informative segments based on their information content determined using segment scoring matrices. Then, using exact methods, we construct a global msa involving only the informative segments. Finally, using approximate methods we construct the alignment of non-informative segments and stitches them into the alignment of informative segments.

**Remark:** The statistical theory for evaluating alignments in terms of its information content was developed for local alignments by Karlin and Altschul [23]. However, their theory do not extend to the case of global alignments. The pair-HMMS provide a framework for statistical analysis of pair-wise global alignments for complex scoring schemes using standard methods like Baum-Welch and Viterbi training. However, determining the right set of parameters for optimal statistical support is highly non-trivial and involves dynamic programming algorithms with computational complexity that is quadratic in the length of the given sequences.

The rest of this paper is structured as follows. In Section 2, we define the problem and introduce the relevant terms and notations to define our segment scoring schemes and heuristics. In Section 3, we present our segment scoring schemes. In Section 4, we present our heuristics, in Section 5, we describe our experimental set-up and summarize our preliminary experimental results, and in Section 6, we present our conclusions and future work.

## 2. Preliminaries

In this section, we first define the problem of msa construction for a given a set of sequences and their segment decompositions, where each segment is classified into one of many types and is associated with a weight reflecting its importance relative to other segments within that sequence. Then, we introduce some basic terms and definitions that are required for defining our scoring models and heuristics.

### 2.1 Problem Definition

Let  $\mathcal{S} = \{ S_1, \dots, S_k \}$  be a set of  $k$  related protein sequences each of length  $n$ . For  $i \in [1..k]$ , let  $B_i = \{ B_i^1, \dots, B_i^{n_i} \}$  be the decomposition of  $S_i$  into  $n_i$  segments. Each segment  $s \in B_i$  is classified into one of many types based on the type of features that are known/predicted to be present in that segment, and is associated with a non-negative real number weight that reflects the importance of the feature associated with that segment relative to other features in that sequence. That is, each segment  $s \in B_i$ ,  $i \in [1..k]$ , is associated with a type  $type(s)$ , and a non-negative real number weight  $weight(s)$ .

**Example:** If the sequences in  $\mathcal{S}$  are partitioned into segments based on their predicted secondary structure, each segment is classified into one of three types *helix*, *strand* or a *coil*, is associated with a non-negative weight in the interval  $[1, 10]$  that reflects the confidence in its secondary structure classification.

Given a set  $\mathcal{S}$  of  $k$  biological related sequences, their decomposition into segments, and the type and weight associated with each of these segments, our goal is to design fast progressive alignment based heuristics that exploit the information content in these segments to build a biologically significant multiple sequence alignment.

## 2.2 Basic Terms and Definitions

Now we introduce some terms and definitions that will necessary for defining our segment scoring models and heuristics.

**DEFINITIONS 2.1** For  $i \in [1..k]$ , we define

- $B_i^{inf} = \{s \in B_i : \text{weight}(s) \geq \alpha\}$  to be the segments in  $S_i$  whose weights are greater than or equal to  $\alpha$ , where  $\alpha$  is a non-negative user specified real number parameter. We refer to the segments in  $B_i^{inf}$  as informative segments of  $S_i$ ;
- $S_i^{inf}$  to be the subsequence of  $S_i$  obtained by concatenating the segments in  $B_i^{inf}$  in the order in which they appear in  $S_i$ . We refer to this subsequence as the informative sequence of  $S_i$ .

**DEFINITION 2.2** For a pair of segments  $s \in B_i^{inf}$  and  $t \in B_j^{inf}$  of the same type,  $i \neq j \in [1..k]$ , we define  $L^H(s, t)$  to be the local alignment between  $s$  and  $t$  constructed using heuristic  $H$  and BLOSUM62 scoring matrix and  $SEG^H(s, t)$  to be bit score corresponding to  $L^H(s, t)$ .

**DEFINITIONS 2.3** For  $i \neq j \in [1..k]$ , we define

- $\alpha_{i,j}$  to be a real number in the interval  $[0, 2]$  that reflects the level of divergence between  $S_i$  and  $S_j$ . We estimate the level of divergence between  $S_i$  and  $S_j$  using the bit score of a local alignment between  $S_i^{inf}$  and  $S_j^{inf}$  constructed using heuristic  $H$  and BLOSUM62 scoring matrix;
- $c : [0 - 2] \rightarrow R^+$  is a function that computes for a given level of divergence the information threshold for an alignment to be informative.

**DEFINITIONS 2.4** For a segment  $s \in B_i^{inf}$  and  $j \neq i \in [1..k]$ , we define

- $Neighbor_j(s) = \{t \in B_j^{inf} : \text{type}(t) = \text{type}(s) \wedge SEG^H(s, t) \geq c(\alpha_{i,j}) * |s|\}$  to be the set of informative segments  $t$  in  $S_j$  of the same type as  $s$  with bit score of a local alignment between  $s$  and  $t$  greater than or equal to  $c(\alpha_{i,j}) * |s|$ . We refer to the segments in  $Neighbor_j(s)$  to be the neighbors of  $s$  in  $S_j$ .
- $Closest - neighbor_j(s) = \{u' \in B_j^{inf} : SEG^H(s, u') = \max_{t \in Neighbor_j(s)} SEG^H(s, t)\}$  is the neighbor of  $s$  in  $S_j$  that maximizes the bit score of a pair-wise local alignment with  $s$ . We refer to such a segment to be the closest neighbor of  $s$  in  $S_j$ .

- $Neighborhood(s) = \bigcup_{j \in [1..k]} Closest - neighbor_j(s)$  to be the set consisting of a closest neighbor of  $s$  from each sequence in  $\mathcal{S}$ .

DEFINITIONS 2.5 For  $i \in [1..k]$ ,

- $B_i^{nei} = \{s \in B_i : s \text{ is a neighbor of some segment in } \mathcal{S} \setminus S_i. \}$ .
- $S_i^{nei}$  to denote the subsequence obtained by concatenating the segments in  $B_i^{nei}$  in the order in which they appear in  $S_i$ . We refer to this subsequence as the neighbor sequence of  $S_i$ .

DEFINITIONS 2.6 For each pair of segments  $s \in S_i^{nei}$  and  $t \in S_j^{nei}$  of the same type from distinct sequences in  $\mathcal{S}$ , and  $l \neq i, j \in [1..k]$ , we define

- $Mutual - neighbors_l(s, t) = \{u \in B_l^{nei} : u \in Neighborhood_i(u) \cap Neighborhood_j(u)\}$  to be the segments in  $S_l$  that are neighbors of both  $s$  and  $t$ .
- $Closest - mutual - neighbor_l(s, t) = \{u' \in B_l^{nei} : SEG^H(s, u') + SEG^H(t, u') = \max_{u \in Mutual - neighbors_l(s, t)} (SEG^H(s, u) + SEG^H(t, u))\}$  to be the mutual neighbor  $u$  of  $s$  and  $t$  in  $S_l$  that maximizes  $SEG^H(s, u) + SEG^H(t, u)$ . We refer to such a segment as the closest mutual neighbor of  $s$  and  $t$  in  $S_l$ .
- $Mutual - neighborhood(s, t) = \bigcup_{j \in [1..k]} Closest - mutual - neighbor_j(s, t)$  to be the set consisting of a closest mutual neighbor of  $s$  and  $t$  from each sequence in  $\mathcal{S}$ .

### 3. Scoring Models for Global Segment Alignment

In this section, we define scoring models for pair-wise segment alignment of sequences. We classify segments into informative and non-informative based on their weight and construct segment scoring matrix entries only for informative segments. Restricting the segment scoring matrix entries to only informative segments helps to significantly reduce the computational time of our heuristics with minimal impact on alignment accuracy. In Section 3.1, we introduce scoring schemes for aligning pairs of informative segments. In Section 3.2, we introduce scoring schemes for aligning a segment with a gap.



### 3.1 Scoring Schemes for Aligning Pairs of Segments

We now introduce the following three scoring schemes for aligning a pair  $s, t$  of informative segments of the same type: (i) Progressive scoring; (ii) Linear Consistency scoring, and (iii) Quadratic Consistency scoring.

**Progressive scoring:**  $SCORE(s, t) = SEG^H(s, t)$ . In this scheme, we only make use of the information content of a pair wise local alignment between segments  $s$  and  $t$  constructed using heuristic  $H$  and BLOSUM62 scoring matrix.

**Linear consistency scoring:**

$SCORE(s, t) = |Mutual - neighborhood(s, t)| * \sum_{u \in Mutual - neighborhood(s, t)} (SEG^H(s, u) + SEG^H(t, u))$ . In this scheme, we make use of the information from both (i) pair-wise local alignment between  $s$  and  $t$ , and (ii) pair-wise alignments involving the segments  $s$  and  $t$  with segments in their mutual neighborhood.

**Quadratic consistency scoring:**

$SCORE(s, t) = |Mutual - neighborhood(s, t)|^2 * \sum_{u \in Mutual - neighborhood(s, t)} (SEG^H(s, u) + SEG^H(t, u))$ . In this scheme, the information obtained through the alignment of two conserved segments of two diverging sequences is weighed more than the information obtained through the alignment of two non-conserved segments of two closely related sequences.

### 3.2 Scoring Schemes for Aligning a Segment with a Gap

We now introduce the following two scoring schemes for aligning an informative segment with a gap: (i) Zero gap penalty, and (ii) Maximum gap penalty.

**Zero gap penalty:**  $SCORE(s, -) = 0$ . In this scheme, we do not penalize the deletion of any informative segment.

**Maximum gap penalty:**  $SCORE(s, -) = \max_{t \in Neighborhood(s)} SEG^H(s, t)$ . In this scheme, the gap penalty of  $s \in B_i$  based on the informative segment  $t \in \mathcal{S} \setminus \mathcal{S}_i$  of the same type that maximizes the bit score of a pair-wise local alignment between  $s$  and  $t$ .

## 4. Heuristics for msa Construction

We now present a generic framework for designing template based fast progressive alignment heuristics that construct global msa as follows:

- (i) construct  $DIST^{nei}$ , a matrix of pair-wise sequence distances, based on scores of pair-wise global segment alignment involving only the informative segments;
- (ii) construct a guide tree  $G^{nei}$  using  $NJ$  algorithm using  $DIST^{nei}$  and build  $MSA^{nei}$ , a msa of the informative segments, by progressively pair-wise segment aligning sequences consistent with  $G^{nei}$ ;
- (iii) construct the pair-wise global alignment of the residues in non-informative segments using fast approximate methods and stitch them back into  $MSA^{nei}$ .

In Section 4.1, we describe our heuristic and in Section 4.2, we present our Heuristic A.

### 4.1 Description of Our Heuristic

**Construction of pair-wise sequence distances:** We now describe how we compute the pair-wise sequence distances for each pair of sequences in  $\mathcal{S}$ .

**DEFINITIONS 4.1** For  $i, j \in [1..k]$ , we define

- a global segment alignment *between two sequences  $S_i$  and  $S_j$  to be an alignment where a segment in  $S_i$  is either aligned to a gap or another segment in  $S_j$  of the same type;*
- $G^{nei}(i, j)$  *to be the optimal global segment alignment between  $S_i^{nei}$  and  $S_j^{nei}$  constructed using the segment scoring matrix  $SCORE$ ;*
- $DIST^{nei}(i, j)$  *to be the score corresponding to  $G^{nei}(i, j)$ .*

Notice that if each segment consists of a single amino acid then the global segment alignment is the same as a traditional global alignment. In this case, the traditional scoring matrices can be used to score alignments between segments. Otherwise, one needs to determine an appropriate segment scoring matrix and then using Needleman-Wunsch's [32] dynamic program construct an optimal global pair-wise segment alignment.

**Construction of Guide Tree and msa of Informative Segments:** We construct the

guide tree  $G^{nei}$  from pair-wise sequence distance matrix  $DIST^{nei}$  using the Neighbor Joining (NJ) algorithm. Then, we construct  $M^{nei}$ , the msa of informative of sequences in  $\mathcal{S}$ , by progressively pair-wise globally segment aligning the sequences  $S_1^{nei}, S_2^{nei}, \dots, S_k^{nei}$  consistent with  $G^{nei}$ .

### Stitching the sites in non-informative segments into msa of informative sequences:

We will describe now for each pair of sequences  $S_i$  and  $S_j$  that were progressively aligned while constructing  $M^{nei}$ , how we stitch the alignment of sites in  $S_i$  and  $S_j$  that were either in non-informative segments or in non-aligned portion of informative segments into  $M^{nei}$ . First, we introduce some necessary definitions.

**DEFINITIONS 4.2** For a pair of informative segments  $s \in B_i^{inf}$  and  $t \in B_j^{inf}$  of the same type, let  $L^H(s, t)$  be the local alignment of  $s$  and  $t$  constructed using heuristic  $H$  and BLOSUM62 matrix. we now define

- $PREFIX_s(L^H(s, t))$  to be the prefix of segment  $s$  that is not part of the local alignment  $L^H(s, t)$ ;
- $SUFFIX_s(L^H(s, t))$  to be the suffix of segment  $s$  that is not part of the local alignment  $L^H(s, t)$ .

Let  $G^{nei}(i, j), i \neq j \in [1..k]$ , denote an optimal global segment alignment between sequence  $S_i^{nei}$  and  $S_j^{nei}$  constructed using the segment scoring matrix  $SCORE$ . For  $G^{nei}(i, j)$ , we say a segment  $s \in S_i$  to be a *matched segment* if in  $G^{nei}(i, j)$  it is aligned to a segment  $t \in S_j^{nei}$ , otherwise it is an *unmatched segment*. We now present a procedure *stitch* that stitches the alignment between the sites in  $S_i$  that occur between any two consecutive matched segments  $s$  and  $\hat{s}$  and the sites in  $S_j$  that occur between the corresponding matched segments  $t$  and  $\hat{t}$  into  $G^{nei}(i, j)$ .

**Procedure** *Stitch*( $s, \hat{s}$ ):

- Let  $p, \hat{p} (q, \hat{q})$  be the respective indices of segments  $s, \hat{s} (t, \hat{t})$  in  $S_i$  and  $S_j$ ;
- Let  $A = SUFFIX_i(B_i^p, B_j^q) \cup_{l=p+1}^{\hat{p}-1} B_i^l \cup PREFIX_i(B_i^{\hat{p}}, B_j^{\hat{q}})$  be the sequence of sites in  $S_i$  that are either in non-informative segments or unaligned portions of informative segments in  $G^{nei}(i, j)$ ;

- $B = SUFFIX_j(B_i^p, B_j^q) \cup_{l=q+1}^{\hat{q}-1} B_j^l \cup PREFIX_j(B_j^{\hat{p}}, B_j^{\hat{q}})$  be the sequence of sites in  $S_j$  that are either in non-informative segments or unaligned portions of informative segments in  $G^{nei}(i, j)$ ;
- Globally align segments  $A$  and  $B$  using BLOSUM62 scoring matrix and any fast linear time heuristic and then insert this alignment between segments  $s$  and  $\hat{s}$  in  $G^{nei}(i, j)$ .

## 4.2 Heuristic $A(\alpha, H, c)$

Parameters:

- (1)  $\alpha$ : a non-negative real number;
- (2)  $H$ : an algorithm/heuristic for pair-wise local alignment of sequences;
- (3)  $c$ : a function that maps for any given level of divergence in the interval  $[0, 2]$  to the information threshold for an alignment to be informative.

Inputs:

- (1)  $S = \{S_1, \dots, S_k\}$ : the set of  $k$  input sequences;
- (2)  $B = \{B_1, \dots, B_k\}$ : the set consisting of the segment decompositions of the sequences in  $\mathcal{S}$ , where each segment  $s$  is associated with a type  $type(s)$  and weight  $weight(s)$ ;

Main Heuristic

- (1) For  $i \in [1..k]$ , construct  $B_i^{inf} = \{s \in B_i : weight(s) \geq \alpha\}$  and  $S_i^{inf}$  the sequence of informative segments.
- (2) For each pair of informative segments  $s \in B_i^{inf}$  and  $t \in B_j^{inf}$  of the same type, using heuristic  $H$  and BLOSUM62 scoring matrix construct  $L^H(s, t)$  and compute  $SEG^H(s, t)$ .
- (3) For  $i \neq j \in [1..k]$ , set  $\alpha_{i,j}$  to the bit score per unit length corresponding to  $L^H(S_i^{inf}, S_j^{inf})$ , the local alignment between  $S_i^{inf}$  and  $S_j^{inf}$  constructed using heuristic  $H$  and BLOSUM62 scoring matrix.
- (4) For each informative segment  $s \in B_i^{inf}$  and  $j \neq i \in [1..k]$ , compute the following:
  - (i)  $Neighbor_j(s) = \{t \in B_j^{inf} : type(t) = type(s) \wedge SEG^H(s, t) \geq c(\alpha_{i,j}) * |s|\}$ .

- (ii)  $Closest-neighbor_j(s) = \{u' \in B_j^{inf} : SEG^H(s, u') = \max_{t \in Neighbor_j(s)} SEG^H(s, t)\}.$
- (iii)  $Neighborhood(s) = \bigcup_{j \in [1..k]} Closest-neighbor_j(s).$
- (5) For each sequence  $S_i \in \mathcal{S}$ ,  $i \in [1..k]$ , construct  $B_i^{nei} = \{s \in B_i : s \text{ is a neighbor of some segment in } \mathcal{S} \setminus S_i.\}$  and  $S_i^{inf}$ , the neighbor sequence of  $S_i$ .
- (6) For each pair of segments  $s \in S_i^{nei}$  and  $t \in S_j^{nei}$  from distinct sequences and  $i \neq j$ ,  $i, j \in [1..k]$ , compute
  - (i)  $Mutual-neighbor_{s_l}(s, t) = \{u \in B_l^{nei} : u \in Neighbor_i(u) \cap Neighbor_j(u)\}.$
  - (ii)  $Closest-mutual-neighbor_l(s, t) = \{u' \in B_l^{nei} : SEG^H(s, u') + SEG^H(t, u') = \max_{u \in mutual-neighbor_{s_l}(s, t)} (SEG^H(s, u) + SEG^H(t, u))\}.$
  - (iii)  $Mutual-neighborhood(s, t) = \bigcup_{j \in [1..k]} Closest-mutual-neighbor_j(s, t).$
- (7) For each segment  $s \in B_i^{nei}$ ,  $i \in [1..k]$ , compute  $SCORE(s, -)$ .
- (8) For each pair of segments  $s \in B_i^{nei}$  and  $t \in B_j^{nei}$  of the same type, compute  $SCORE(s, t)$ .
- (9) For  $i \neq j \in [1..k]$ , compute  $DIST^{nei}(i, j)$  by globally segment aligning  $S_i^{nei}$  and  $S_j^{nei}$  using Needleman-Wunch's dynamic program and segment scoring matrix  $SCORE$ .
- (10) We now construct the msa of  $\mathcal{S}$  as follows:
  - (i) Construct guide tree  $T^{nei}$  from  $DIST^{nei}$  using the Neighbor Joining (NJ) algorithm.
  - (ii) Construct  $M^{nei}$  by progressively globally segment aligning the sequences  $S_1^{nei}, \dots, S_k^{nei}$  a pair at a time consistent with  $T^{nei}$ .
  - (iii) For each pair of sequences  $S_i^{nei}$  and  $S_j^{nei}$  that were progressively aligned while constructing  $M^{nei}$ ,
    - Let  $G^{nei}(i, j)$  denote the global segment alignment of  $S_i^{nei}$  and  $S_j^{nei}$ ;
    - For each pair  $s, \hat{s}$  of consecutive matched segments of  $S_i^{nei}$  in  $G^{nei}(i, j)$  (where  $t, \hat{t}$  are the corresponding matched segments of  $S_j^{nei}$ ) use *procedure stitch* to stitch the alignment between the sites in  $S_i$  that occur between  $s$  and  $\hat{s}$  and the sites in  $S_j$  that occur between the sites in  $t$  and  $\hat{t}$  to  $G^{nei}(i, j)$ .

## 5. Experimental Results

In this section, we first describe our experimental set-up, then we describe how we evaluate the performance of our heuristic, and finally we summarize our preliminary experimental results.

### 5.1 Experimental Set-up

Our computational experiments have been set-up with the focus on analyzing the performance of our heuristics for sequences from protein families in the PFAM [13] database for which (i) accurate reference alignments were available either through structural aligners or through other sequence independent biological methods, and (ii) annotations describing the salient biological features were available for each sequence. We chose 12 sets of sequences ranging from 5 to 23 sequences with sequence similarity ranging from 20% to 80%. For these sequences, we used PSIPRED [22], a widely used structure prediction tool, to partition each sequence into segments based on their secondary structure characteristics. PSIPRED classifies each segment into one of three types *helix*, *strand* or a *coil*, and associated a non-negative weight in the interval  $[1, 10]$  reflecting the confidence in its partitioning and classification. Then for these sets of sequences, we construct an msa by using our heuristic  $A(\alpha, H, c)$ , where  $\alpha$  is a non-negative real number parameter for classifying segments based on their weights into informative and non-informative segments,  $H$  is an algorithm/heuristic for pair-wise local alignment of segments, and  $c$  is a function that maps for any given level of divergence in the interval  $[0, 2]$  to the information threshold for an alignment to be informative. In our experiments, we have set  $\alpha$  to be 6. That is a segment is considered to be informative if its average segment weight  $\geq 6$  (i.e.  $\alpha \geq 6$ ) and its length is at least 5. In addition, if two informative segments of the same type are separated by less than 4 residues we merged the two segments with the intervening residues into a single informative segment. We set  $H$ , the algorithm/heuristic for local alignment to be BLASTP [1, 30] with slight modifications to handle alignments involving short sequences.<sup>1</sup> We defined the function  $c$  based on the average bit scores of BLOSUM matrices corresponding to different levels of sequence divergence.

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<sup>1</sup>The quality of alignment constructed using Smith-Watermans dynamic program was not significantly different from that obtained using BLASTP.

## 5.2 Evaluation the Performance of Our Heuristic

We evaluate the performance of our heuristic based on (i) the accuracy of its msa in comparison with an reference alignment, and (ii) its computational efficiency for the appropriate choice of its parameters  $\alpha$ ,  $H$  and  $c$ ,

**Evaluating accuracy of an msa:** The traditional sequence similarity based measures like SP score and Tree score have only been helpful in providing a crude estimate of the alignment quality and measures based on structurally correct alignments are likely to be better alternatives for evaluating alignment accuracy. So, for sequences for which their 3D structure is known, the accuracy of an msa can be evaluated in comparison with reference alignments constructed through a structure aligner. We also observe instances of homologous sequences that share only a few features and yet preserve their overall structure and function. In these instances, local feature conservation is another good predictor of alignment accuracy. So, we measure the accuracy of the msas constructed by our heuristic in terms of the percentage correlation between the columns in the multiple sequence alignments constructed by our heuristic and the columns of the sites within the reference alignment that correspond to conserved features.

**Note:** Our heuristics make use of the secondary structure predictions from PSIPRED. So, any inaccuracies in the secondary structure prediction of PSIPRED should also be factored while evaluating the accuracy of msa constructed by our heuristics. We factor this in terms of the correlation between the informative sites in our heuristic and the sites in the reference alignment that correspond to conserved features. We also restrict the impact of inaccuracies in secondary structure prediction on msa accuracy by conservative choice of the information threshold function  $c$  (i.e. higher than if we had an accurate partitioning and correct classification of segments).

**Evaluation of Computational Efficiency:** Our heuristics attempt to minimize its computational time with minimal impact on its accuracy by first classifying the segments within each sequence into informative (non-informative) segments based on its weight exceeding (not exceeding)  $\alpha$ . Then, the msa is essentially constructed by first progressively pair-wise aligning the sites in informative segments using exact methods and then us linear time ap-

proximate heuristics to align the sites in non-informative segments and stitch them back into the alignment of sites in informative segments. So, the saving in computational time depends on the fraction of the segments that are informative. This in turn depends mainly on the choice of the information threshold  $\alpha$ .

### 5.3 Summary of Preliminary Experimental Results

Protein Family	# of Sequences	Sequence Lengths	% Sequence Similarity	# of informative segments	Avg Length of informative segment	% Local Similarity
PF13420	21	152-164	20%-70%	4	10	< 70% <sup>a</sup>
PF13652	11	131-152	65%-85%	4	12	> 90%
PF13693	22	77-81	55%-83%	3	12	> 90%
PF13733	5	133-142	55%-61%	2	8	> 90%
PF13844	6	449-481	68%-78%	7	12	> 90%
PF13856	23	90-112	30%-73%	3	10	> 80% <sup>a</sup>
PF13944	21	120-146	30%-85%	3	10	> 90%
PF14186	11	152-157	38%-68%	4	8	> 90%
PF14263	10	120-129	50%-66%	3	10	> 90%
PF14274	20	155-165	36%-71%	3	12	> 90%
PF14323	18	485-548	36%-43%	6	11	> 90%

<sup>a</sup>: Quadratic Consistency and Max Gap Penalty Scoring Schemes was employed.

Table 1: Summary of msa results using Linear Consistency and Max Gap Penalty Schemes

## 6. Conclusions and Future Work

Our preliminary experimental results indicate that our template based heuristic framework can help in designing heuristics that can exploit template based information to construct msas that are biologically accurate in a computationally efficient manner. However, we would like to (i) make use of *extreme value distribution* [16] to define the the function  $c$  that maps for a given level of sequence divergence the information threshold for an alignment to be informative; (ii) Understand how to define the segment scoring schemes for aligning sequences that are highly divergent; (iii) evaluate the accuracy of the alignments constructed by our heuristics by using sequence independent measures [2, 25, 34] on challenging datasets in BALiBASE [27, 28].



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